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Assessment of thin-film photocatalysis inactivation of different bacterial indicators and effect on their antibiotic resistance profile

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ABSTRACT

The presence of bacterial pathogens in water bodies, alongside their growing antibiotic resistance, endanger access to freshwater sources and necessitate their successful inactivation with a proper disinfection technology. In the present study, a parallel plate reactor (PPL) with immobilized photocatalyst was used as a disinfection system for the inactivation of two bacterial indicators (*Escherichia coli* and *Enterococcus faecalis*) in aqueous samples. Experiments were carried out at parallel plate reactor configuration (PPL) operated in recycling batch mode. Titanium Tetraisopropoxide (TTIP) based thin-film coated photocatalyst surfaces were used and assessed operations parameters were; pH, initial bacteria concentration, source and type of bacteria. The effect of the photocatalytic process on antibiotic resistance profile of target bacteria was also investigated as it may serve as a pioneering step in the field of well-established and modern disinfection method development, without causing proliferation of antibiotic resistance. The observed courses of bacterial inactivation and the final disinfection rates point out diversity in the level of interaction between different type/source of bacteria and photocatalyst of concern. According to the results, a stationary phase of bacteria inactivation proceeded with a rapid and efficient one for the case of *E. faecalis* (99% removal after 180 min and 99.9% removal after 240 min), while the trend for *E. coli* is more likely to be described as extended along the process time. Among the tested antibiotics, *E. coli* Minimum inhibition concentration (MIC) values for beta-lactam, macrolide and aminoglycoside groups were considerably altered (namely Ampicillin, Cefaclor, Clarithromycin - Erythromycin and Amikacin, respectively). PC oxidation was approved to be efficient on bacterial inactivation and trigger alterations on resistance behaviour of *E.coli* and *Enterococcus* sp. strains.

1. Introduction

The excessive use of antibiotics and their significant presence in sewage have been reported to proliferate ARB. The dispersion of ARB (antibiotic resistant bacteria) into the aquatic environment seems to be of major concern, as they promote antibiotic resistance spread [1,2]. [3,4]. Being embedded in the genetic mobile platforms (plasmids, transposons, integrons), antibiotic resistance genes emerge from hosting bacteria. At this point, natural bacterial ecosystem in soil and water constitute a way of resistance spread, as they both take primary role in biological water treatment systems. Diversity of mechanisms responsible for the bacteria resistance behaviour and number of resistance genes characterized in both Gram-positive and Gram-negative bacteria necessitates further evaluation of resistance profile following the disinfection treatment [1,5].

Among the various advanced oxidation processes (AOPs), photocatalysis (PC) has become a promising sustainable green photochemical process that provide acceptable levels of bacterial inactivation [6–8]. In relation to its cost, chemical and photochemical stability, availability and reduced toxicity in the environment, TiO_2 is the most widely used photocatalyst [9,10]. There is precedence of reactors using immobilized catalysts systems for environmental applications. Durability, requirement for catalyst separation are parameters of concern by which a featuring process become prominent amongst alternatives [11–13]. There are also numerous applications in the field of solar bacteria inactivation at different reactor configurations. In the sense of process efficiency, tubular reactor configurations supported with compound parabolic collectors are one step ahead. Concentric tube configurations

Abbreviations: ARB, antibiotic resistant bacteria; AMK, amikacin; AMP, ampicillin; CEC, cefaclor; CFU, colony forming unit; CLR, clarithromycin; ERY, erythromycin; LVX, levofloxacin; MIC, minimum inhibitory concentration; PC, photocatalytic; PPL, parallel plate; ROS, radical oxygen species; SMX, sulfamethoxazole; UV, ultraviolet; TF, thin-film

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are reported to be the most efficient onex amongst different tubular configurations in the field of solar disinfection [14]. The recycling batch mode of operation in a parallel plate (PPL) reactor configuration was reported to provide replicable experimental results on degradation of both micro-pollutants and bacteria inactivation [12,15]. The PPL reactor configuration enables shifting the reaction medium of slurry reactors to two dimensions and enables simple alterations in process/reactor conditions such as; the illuminated total volume ratio, flow rate and catalyst load, which are listed amongst the main factors affecting PC treatment efficiency [16,17].

Despite the fact that ultimate goal of those procedures are mostly complete bacterial inactivation of bacteria, many of them remain active after treatment with variable response in the presence of antibiotics [18]. Given their resistance to various stress conditions and persistence properties, inactivation of bacteria requires development of a satisfactory disinfection technology. Research on state of the art disinfection technologies must take into account the changes in antibiotic resistance (AR) profile of the target species. With this regard, the objectives of this study are:

i) The application of Thin-film (TF) photocatalytic disinfection at PPL reactor configuration, in order to investigate inactivation rates of reference and environmental bacterial strains of *Escherichia coli* and *Enterococcus* sp.;
ii) Determining the best fitting kinetic model considering the variations on the type and source of target bacteria;
iii) Determining the alterations in antibiotic resistance profiles of non-treated and intact cells (exposed to PC treatment) for Beta-lactam, macrolide, aminoglycoside and sulphonamide groups of five antibiotics namely Ampicillin, Cefaclor, Clarithromycin- Erythromycin and Amikacin, respectively.

From above mentioned aspects, this study has set its goal on determining wide range applicability and bacteria inactivation rates of thin-film photocatalysis at optimal PPL reactor conditions. In addition, alterations on the antibiotic resistance profile of PC treated bacteria cells have been evaluated.

2. Materials and methods

2.1. Chemicals and solutions

Amikacin (AMK) (CAS# 37517-28-5), Ampicillin (AMP) (CAS# 69-52-3:, Cefaclor (CEC) (CAS# 53994-73-3), Clarithromycin (CLR) (CAS# 81103-11-9), Erythromycin (ERY) (CAS# 114-07-8), Levofloxacin (LVX) (CAS# 100986-85-4) and Sulfamethoxazole (SMX) (CAS# 723-46-6) were purchased from Sigma-Aldrich at the highest purity (> 99%). 1.Ultra-pure water was used for all experiments. The device was Direct-Q3 water purification system (Merck Millipore). The pH adjustment was made with dropwise addition of 001 N NaOH and HCl solutions keeping the rate of dilution below %0.1-0.5 interval.

2.2. Thin film & PPL reactor

Polyethylene glycol enriched Titanium Tetraisopropoxide based TFs were fabricated and used in the PC experiments. Preparation and characterization of thin-films have been described in our previous study [19]. The PPL reactor operated in fully plug-flow conditions with no recirculation or dead-zones along the flow direction. As indicated in the literature the PPL model is one of the most efficient configuration for water treatment. The UV illumination was provided by three Philips-BLB 8 W lamps positioned on the top of PPL reactor. The configuration was set to provide UV-A energy equal to 0.90 mw/cm^2 [12,19].

2.3. Inoculum and sample preparations in photocatalytic disinfection processes

Under disinfection experiments, *E. coli* DSM-498 and *E. faecalis* ATCC-14506 were used as bacterial reference strains. Experimental runs were conducted with samples consisted of sterilized water with 0.8 w/v % NaCl. All samples were inoculated with the required volume of *E. coli* or *E. faecalis* liquid culture, grown overnight at 37 °C in nutrient broth (LABM). The initial bacterial population was set between 10^3–10^7 CFU/mL. The estimation of bacterial concentration in suspensions was performed measuring the optical density of the liquid culture at 600 nm (Shimadzu UV1240 spectrophotometer) and using the McFarland scale [22].

2.4. Isolation of environmental bacterial strains

Environmental bacterial strains of *E. coli* and *Enterococcus* sp. were isolated from seawater samples taken in Chania, Greece. Bacterial isolation was performed by filtration of samples through nitrocellulose membranes (0.45 µm pore size, 47 mm diameter, Pall-Gelman Laboratory) followed by culture on selective media. HiCrome Coliform Agar (HiMedia Laboratories) incubation at 37 °C for 24 h and Slanetz & Bartley medium (LABM) incubation at 37 °C for 48 h were culture conditions for *E. coli* and *Enterococcus* sp. respectively. Inoculation of *Enterococci* bacteria was performed by transferring the membranes onto Bile-Aesculin-Azide agar (LABM), preheated at 44 °C.

2.5. Disinfection experiments

In each photocatalytic experimental run, the initial samples (the aqueous sample inoculated with the desired bacterial concentration) with 500 mL volume were kept in a glass flask (feeding tank) under continuous stirring. The recirculation of bacterial solution along the PPL reactor was provided with a peristaltic pump. A 90 mL/min of flowrate was set in order to provide an average liquid speed of 1.5 cm/s over the photo-catalyst suface. After each experiment the feeding tank, PPL reactor and pipeline connections were sterilised with 70%v/v ethyl alcohol (S), rinsed with sterile ultrapure water (R) and finally dried with airflow (D). During PC runs, samples were collected at specific time intervals in order to determine the inactivation rate of the bacterial indicators. The culture technique and the serial dilutions of the reaction solution were performed by sterile 0.8% (w/v%) NaCl aqueous solution and streaking 100 µL of each dilution onto the agar plates (NABM). Specifically, bacterial cultures technique was performed in triplicate and the minimum detectable colonies number was found approximately 2–3 CFU/mL (a reflection of the fact that 0.3 mL of each sample: 3 × 100 µL was plated on the agar) Bacterial viable counts were determined through colonies enumeration after incubation at 37 °C for 20–24 h.

Replicability of experiments were determined and standard deviation of each 3 replicates found to be in the 2–5 % interval for each experimental condition (Indicative of an acceptable level of variation in number of surviving bacteria following PC treatment – data not shown) [33,34]. For each different experimental condition, unused thin-films with identical surface property and PC activities were preferred. Accordingly the TF surface regeneration was applied between experimental runs as it was detailed in a recent study of ours [12].

Disinfection rates were estimated using widely reported disinfection models, namely the Chick-Watson, Hom and Modified Hom. All models were fitted to the experimental data *via* a non-linear approach, where MATLAB $f_{minsearch}$ solver function (minimum of unconstrained multivariable function using derivative-free method) was used to estimate model kinetic coefficients for Modified Hom (k_1, k_2, k_3). Bacterial inactivation rates were compared taking into account the log-linear inactivation phases of each scenario [13,23].

Chick has built the first kinetic model on a k coefficient based on

disinfectant dosage, type of microorganism and water matrix conditions and Watson has upgraded the equation with a new coefficient descriptive of the disinfection efficiency [24,25]. Their approach was integrated and named as Chick-Watson disinfection model (1)

$$\log\left(\frac{C}{C0}\right) = kC^{n}t \quad (1)$$

On the other hand, Hom has offered a new bacteria inactivation model. It has targeted determination of bacteria inactivation kinetics which are far from presenting log-linear behaviour [26] (2).

$$\log\frac{C}{C_0} = -k't^{h} \quad (2)$$

Hom model is not the best tool for description of kinetics that present both linear and non-linear bacteria inactivation. Thus a modified version of the Hom model has been offered (3) with the defined coefficients k_1, k_2, k_3 which are representative of initial delay, log-linear phase of inactivation and decay of inactivation rate at last period of inactivation, respectively [27].

$$\log\frac{C}{C_0} = -k_1[1\text{-}\exp(-k_2t)]^{k3} \quad (3)$$

2.6. Antibiotic resistance

Antibiotic resistance assesment was performed on intact (untreated) and residual bacterial colonies after PC treatment. The broth microdilution methodology was applied and MIC of antibiotics were measured as detailed under Section 2.1. MIC values were estimated testing different concentrations of selected antibiotics over *E. coli* and Enterococci liquid cultures (prepared with Mueller-Hinton broth of Merck). Incubation took place at 37 °C for 18–24 h, followed by optical density measurement at 630 nm, using a microplate reader (Labtech LT-4000 Plate Reader) and Manta LML software. The range of antibiotic concentrations were set based on EUCAST (European Committee on Antimicrobial Susceptibility Standards) MIC database and are shown in Table 1

2.7. Optimization of process parameters

Initially, preliminary experiments with *E. coli* DSM-498 bacteria strain were performed. Basic process parameters; UV energy level, initial NaCl concentration were optimized. And replicability of inactivation performances was determined. Effect of the solvent matrix properties on PC activity (organic-inorganic ions) have been well addressed and documented in literature [21,23]. To study the effect of NaCl on bacteria inactivation, varying initial NaCl concentrations (0.008% to 0.8% on w/v basis were to be tested. UV irradiation itself (UV-A and especially UV-C) have been reported to cause cell damage on bacteria. Maximum UV-A energy that can be applied on PC runs has been optimized in order to eliminate effect of photolysis. Flow rate dependency of TF PC bacteria inactivation was tested at laminar flow conditions through PPL reactor.

3. Results and discussion

3.1. Preliminary experimentation

UV-A energy of 0.90 mw/cm^2 was found to be the optimum value for the elimination of the tested bacterial strains beyond the process of photolysis. The ion concentration value of 8 g/L is well above the minimum value that is reported to inhibit PC activity [28–30]. Since photolysis at NaCl 0.8-0.008 w/v% conditions did not lead to significant level of inactivation following a 4 h of UV-A exposure. The physical stress caused by lack of NaCl was not determined as a decisive factor on bacteria survival for the case of studied bacteria strains and experimental conditions (data not shown). Increased flow rate ended with decreased PC bacteria inactivation rates, so it is for the case of decreased flow rate (data not shown).

pH is not only effective on bacteria metabolism but also has a predetermining role on the mechanism of interaction between bacteria cells and photo-catalyst surface [35]. The pH value of 5 was found to be the most suitable condition, considering both metabolic activity of non-irradiated bacteria cells and efficiency of photocatalytic activity under UV-A irradiation (Fig. 1). On the other hand, neutral pH condition of distilled water/ultrapure water or treated/untreated urban waste water samples without prior optimization was most commonly reported in the literature [13,36]. Without UV-A irradiation induced formation of ROS, the photocatalyst coated surface itself was unable to provide significant level of bacteria inactivation under dark (UV-A off) experimental conditions (data not shown).

The predominating phenomenon for bacteria inactivation by surface immobilized PC system is to be adhesion (interaction between surfaces adsorbed bacteria cell and ROS, mostly OH radicals. It is directly related with surface charge properties and the hydrophilic/hydrophobic transition of photo-catalyst under UV irradiation [28,29,31]

3.2. Effect of bacteria type and source on PC inactivation rate

According to applied treatment conditions and source of target bacterial strain, PC bacteria inactivation mechanism may follow a different pathway on Gram (+) and Gram (-) bacteria as can be seen in Fig. 3a. [37]. Compared to Gram (+) bacteria, cell structure is reported to be more complex for Gram (-) bacteria. Thereby, Gram (-) bacteria is inactivated less efficiently compared to Gram (+) which can be attributed to unnecessity of cell wall destruction for complete inactivation of those species [21].

Opposite to the general trend, inactivation of Gram (+) bacteria is more efficient under varying stress conditions compared to Gram (-) bacteria, that is in accordance with experimental results obtained (Fig. 2) [13,21,38]. Besides, in case of exposure to immobilized photocatalyst, PC activity was reported to be higher for the most hydrophobic bacteria. Due to high hydrophilicity of *E. coli*, Gram (-) bacteria that are more hydrophobic compared to *E. coli* or Gram (+) bacteria itself, were reported to show better adherence and interaction with the immobilized catalyst [9,34–39].

The seawater isolated *E. coli* strain has exhibited increased level of resistance to oxidative conditions in comparison with the laboratory

Table 1
MIC values of the selected antibiotics over *E. coli* and *E. faecalis* reported by EUCAST and concentration range of antibiotics studied.

Bacterial species	EUCAST reported MIC values (μg/mL)						
	AMK	AMP	CEC	CLR	ERY	LVX	SMX
Escherichia coli	0.5–8 (4*)	0.25-8 (2*)	0.025–4 (1*)	–	32–512 (32–64*)	0.08–0.25	2–64 (16*)
Enterococcus faecalis	–	0.032-2		0.064 – 1	1–4	0.032-4	–
	Concentration range of the studied antibiotics (μg/mL)						
	0.5–32	0.5–32	16–512	2.5–40	2.5–40	0.5–16	2–32

* The most frequently encountered MIC values within reported interval.

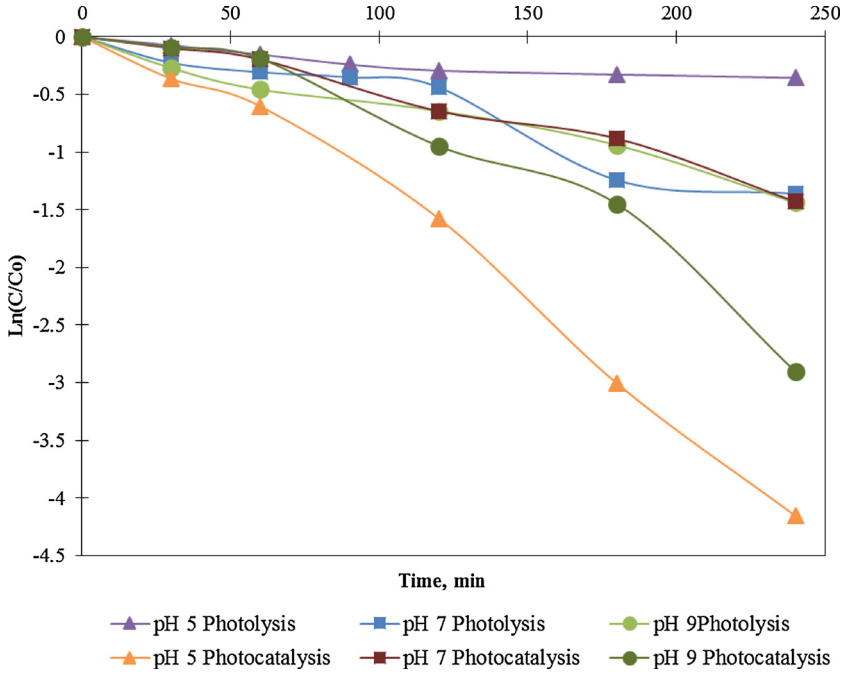


Fig. 1. Photocatalytic-photolytic inactivation of *E. coli* DSM-498 at different pH conditions ; [C] NaCl: 0.08 w/v %, Flow rate: 90 mL/min., Initial [C] $_{bact} = 10^5$ CFU/mL, UV-A energy: 090 mW/cm^2, (Experiments were run as duplicates, mean values are presented while the SE < 5%).

standard strains, while there was no alteration for the case of *Enterococcus sp* (Fig. 2b). For the case of observed differences between PC inactivation of the laboratory and environmentally isolated strains, one should note that diversity of protein structures (their exact positions arising from level, type and duration of exposure to environmental stress in their background) may play a major role in their inactivation mechanism. (Fig. 2b) [34,40]. Based on similar instructions, valid and significant assumptions can be made for different antibiotic resistance profiles exhibited by intact cells and cells belong to post treatment [41].

Log linear inactivation rates were in comparable interval with the reported findings in the studies that used modified HOM model for log linear phase inactivation rate estimation [13,42]. Marugan et al. reported that suspended PC system was able to provide 0.0215-0.030 min^{-1} log linear inactivation rates for sewage isolated ([C]bacteria $=10^5$ to 10^8) *E. coli* ATCC 11775, under UV-A irradiated TiO_2 enhanced kaolinite 6 g/L photocatalyst conditions. While obtained log linear inactivation rates varied between 0.053-0.098 min^{-1} for the case of 1 g/L anatase titanate nanofiber catalyst dose and $[C]_{bacteria} = 3 \times 10^6$ to 1.2×10^7 conditions [23,32,37]

The PC inactivation of *E. faecalis* (Fig. 2b – Table 2) can be justified with enhanced level of interaction between surface adsorbed OH radicals and bacteria cells (Murray et al. 2015; Marugán, van Grieken, Cassano, et al. 2010). In comparison with suspended system alternatives, the TF-PC approved acceptable level of bacteria inactivation in PPL reactor configuration (3–4 Log removal).

3.3. Effect of initial Bacteria concentration

PC inactivation rate of *E. faecalis* was reduced (k_1 from 5.21 to 6.54 and 9.78 by variation of $[C]_{bacteria:}$ 10^3 to 10^7) and the Log-linear phase inactivation rate decreased gradually by 0.1213, 0.159, 0.032 min^{-1} (Table 2 and Fig. 3a) which would fall under the typical shoulder behaviour definition. Rather than exhibiting a shoulder and tail behaviour it is more pronounced as “continuous” inactivation profile for the case of *E. coli* inactivation by PC system. The Log phase inactivation rate has gradually increased over time with the variation of initial *E. coli* DSM-498 concentration from 10^3 to 10^7 (Fig. 3b)

According to Dunlop et.al., (according to homogenous properties of the solution) increases in the initial bacteria concentration bring along enhanced interaction with the catalyst surface which give rise to reduced mass transfer limitations [44]. Similar observation was reported by Chong et al.; Increases in the initial bacteria concentration end up with higher Log inactivation rate for the case of photocatalytic inactivation of *E. coli* ATCC-11775 by anatase titanate nanofiber catalyst [37].

3.4. Effect of photocatalytic treatment on antibiotic resistance profile of bacterial strains

Antibiotic resistance profile was affected to a certain extent, depending mainly on the type of antibiotic and the bacterial strains tested as seen in Table 3 [34,45,46]. The alteration of MIC values after treatment may raise concerns regarding public health, especially in case of bacterial resistance increases. Underlying the non-selective mechanism of PC oxidation through radical oxygen species (ROS) that are present both in the solvent matrix and on the surface of photo-catalyst, porin and protein structure of target bacteria cells are oxidized in a random manner (initial effect) [27,50]. In the case of H_2O_2 and $^{\cdot}OH$ radical formation, both base and ribose moieties of the DNA get oxidized (cell membrane effect] and the cell photo-activation mechanism get almost inactivated [41].

Discussion is underway, whether applied advanced treatment techniques bring along increase of surviving strains resistance to certain antibiotics or the *vice versa*. It is evident from most recent literature findings that the bacterial response (in terms of antibiotic resistance behaviour) to applied treatment may differ by type of antibiotic, bacteria and specific interaction between the microbial cell and the process mechanism [47–51].

Rizzo et al. demonstrated that chlorination is an adequate technique for efficient inactivation but may favour resistance development of regrown bacteria to certain antibiotics [45]. Munir et al also concluded that chlorination might not have significant impact on antibiotic resistance profile *of E. coli* [46].

In this study, the alteration of MIC values as a response to photocatalytic treatment conditions are presented in Table 3. According to MIC determinations for (post PC treatment) survival *E. coli* cells, it is

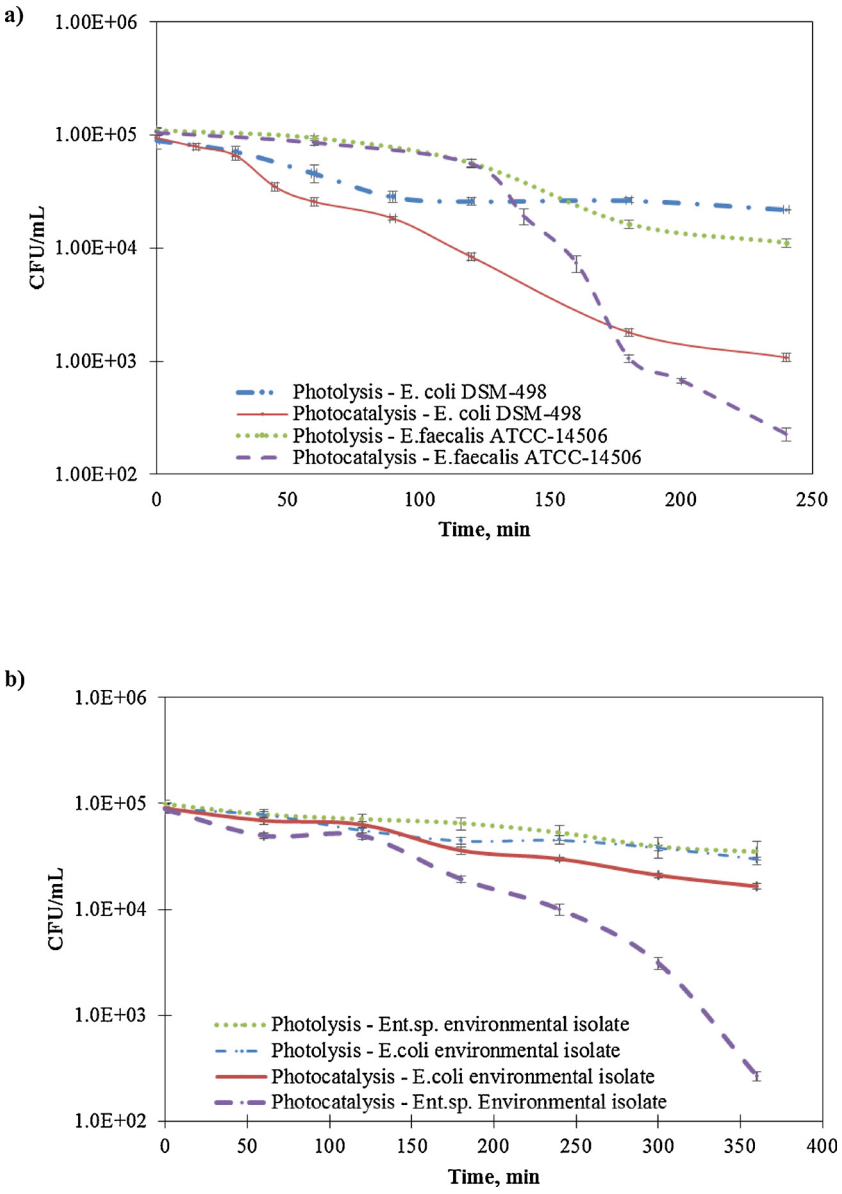


Fig. 2. Photocatalytic inactivation of a) *E. coli* DSM-498 and *E. faecalis* ATCC-14506 b) Environmental isolates of *E. coli* and *Enterococcus* sp. Bacteria. Conditions: Bacteria concentration; 10^5 CFU/mL, $[C]_{NaCl}$: 0.008% w/v, Flow rate: 90 mL/min, UV-A energy: 0.90 mw/cm^2.

evident that bacterial strains have become more susceptible to AMP and AMK antibiotics, while they become more resistant to macrolide group antibiotics and CEC. No MIC alteration has been determined for environmental isolate of *E. coli* except the specific case for macrolide antibiotics; post treatment bacteria cells have become more susceptible to ERY and CLR antibiotics. Besides the obtained level of inactivation (3 LOG reduction at the end of 240 min PC process), antibiotic resistance profile of *E. faecalis* was not affected by the oxidation process and remained stable for most of the antibiotics except the case for CEC antibiotic. The peptidoglycan cell wall *at E. faecalis* is able to protect structural properties and layout of porin and protein structures which play a key role on resistance mechanism towards different environmental and oxidative stress conditions [21].

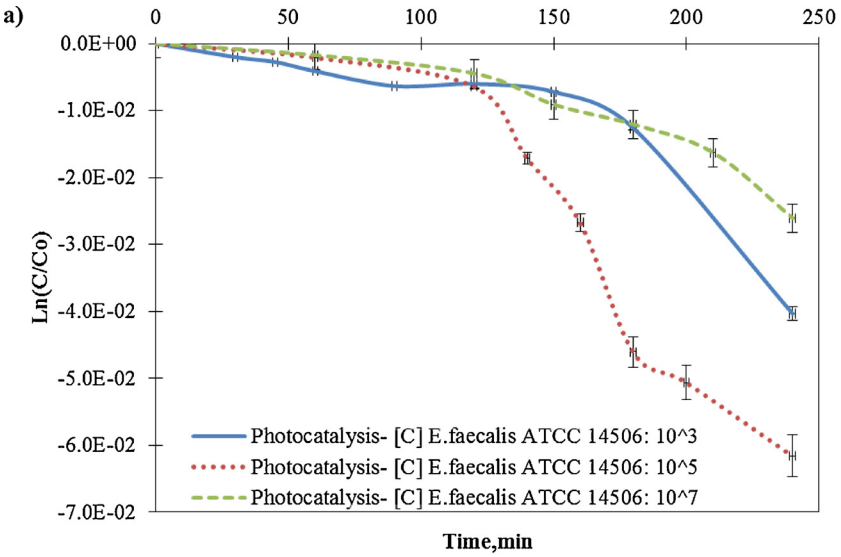
Danae et al. have compared different disinfection methodologies based on obtained inactivation rates and pointed out that process mechanism might cause MIC alterations on survival cells [22]. They have reported that, except the specific case for UV-C, all other treatment options including PC with composite Ti nanoparticles with binary dopants (Mn, Co, Mn-Co) under simulated solar and sun irradiated conditions have led to a decrease of MIC for tetracycline. Resistance level of survival cells to CEC antibiotic remained stable or decreased for the case of PC with Mn-Co binary dopant titanium, while it remained stable or increased after other treatment alternatives [52].

In comparison with the literature findings, the alterations in *E. coli* bacteria resistance profile may be attributed to long treatment periods applied in this study, which was reported to play a determining role on resistance alteration as a result of the process mechanism [51].

4. Conclusion

This study aimed to investigate the wide range applicability of thin-film photo-catalysis at PPL reactor configuration (TF PC) in the field of bacteria inactivation. Different type and sources of bacteria strains exhibited different type and level of response against oxidative conditions resulted from the PC process. The main can be listed as in the following:

- Environmental isolates of *E. coli* show more resistance to oxidative



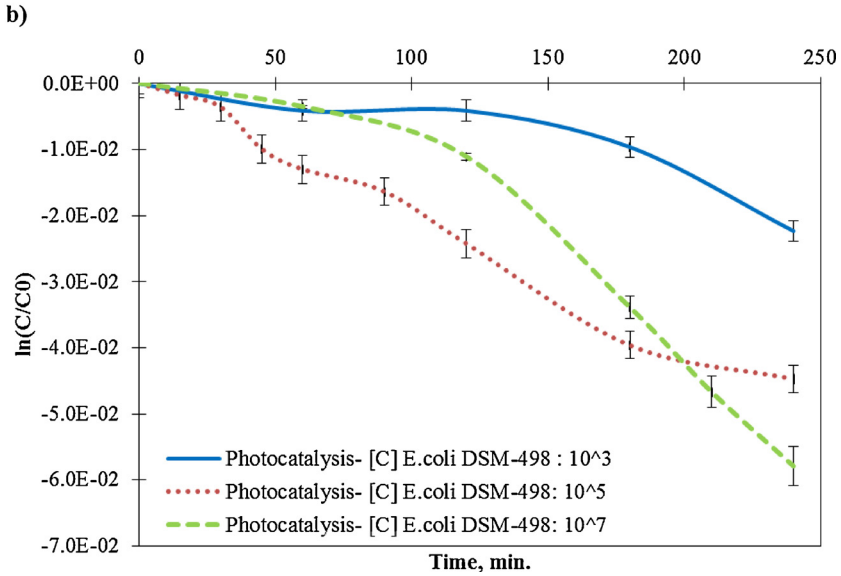


Fig. 3. Photocatalytic inactivation rates for varying initial concentration of *E. coli* DSM-498 and *E. faecalis* ATCC 14506 bacteria. $[C]_{NaCl}$: 0.008% w/v, Flow rate: 90 mL/min, UV-A energy: 0.90 mw/cm^2.

Table 2
Estimated kinetic parameters for the Modified Hom model.

Bacterial strain	Bacteria (CFU/mL)	Coefficients for Modified HOM Model			
		k_1 _ k_3	k_2	RSS	R^2
E. coli DSM-498	10^5	−5.55 to 1.75	0.053	0.114	0.993
E. faecalis ATCC14506	10^5	−6.65 to 45	0.157	0.196	0.995
E. coli Environmental Isolate	10^5	−3.39 to 1.58	0.017	0.035	0.985
Enterococcus sp. Environmental Isolate	10^5	−15.3 to 3.84	0.024	0.911	0.965
E. coli DSM 498	10^3	−15 to 2.42	0.014	0.184	0.972
	10^5	−5.55 to 1.75	0.053	0.114	0.993
	10^7	−6.82 to 6.46	0.073	0.119	0.995
E. faecalis ATCC-14506	10^3	−5.21 to 45	0.121	0.712	0.962
	10^5	−6.54 to 45	0.159	0.176	0.995
	10^7	−9.78 to 4.22	0.032	0.029	0.998

RSS: Residual sum of squares.

Table 3
MIC of the selected antibiotics on the growth inhibition of E. coli and E. faecalis intact and residual cells post treatment; 300 min of photocatalytic treatment, UV-A energy: 0.90 mw/cm$^{2.}$

Bacterial culture-treatment conditions	MIC, μg/mL					
	ERY	CEC	CLR	AMK	AMP	SMX
E. faecalis ATCC 14506 Intact Cells	2.5	32	2.5	0.5	05	2
E. faecalis ATCC 14506 PC	2.5	512	2.5	0.5	0.5	2
E. coli DSM 498 Intact Cells	40	512	40	4	32	16
E. coli DSM 498 PC	20	256	20	1	8	16
E. coli Env. Isolate Intact Cells	20	16	20	0.5	2	8
E. coli Env.Isolate PC	10	16	5	0.5	2	2

conditions compared to standard laboratory strain, *E. coli DSM-498*.
- Initial bacteria concentration is found to be a determining factor but not a limiting one for PC disinfection of *E. coli*.
- Antibiotic resistance profile of intact and post-treatment bacteria cells are indicating that PC oxidation mechanism is also effective on the resistance behaviour of target bacteria.
- Environmental isolates of *E. coli* become less sensitive to changes in antibiotic resistance behaviour following PC oxidation process.

As a result of comparison between rate of PC bacteria inactivation and alterations in antibiotic resistance profile for Gram (-) and Gram (+) bacteria of different sources; it may be concluded that diversity of structural properties bring along variation of resistance to antibiotics and oxidative conditions.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.apcatb.2018.11.095.

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